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QUANTITATIVE HISTOLOGICAL ANALYSIS OF THE EPITHELIUM OF THE VENTRAL SURFACE OF HAMSTER TONGUE IN EXPERIMENTAL IRON DEFICIENCY

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Summary—Quantitative analysis of the ventral tongue epithelium of iron-deficient hamsters revealed significant progressive alterations in epithelial compartment thickness. As iron deficiency developed there was an initial increase in the proportion of the epithelium made up by progenitor cells and a decrease in the proportion formed by maturing cells. With increasing degree of deficiency, the maturation compartment formed less, and the keratinized compartment more, of the epithelium until, at the stage of anaemia, there was a significant reduction in the maturation compartment and a significant increase in the keratin thickness.

INTRODUCTION

Histological studies of oral mucosa in iron deficiency have revealed conflicting results. Jacobs (1960) studying human buccal epithelium in iron-deficiency anaemia found atrophy in some, but not all. Drinnan (1969) studying oral mucosa from iron-deficient rats and mice was unable to demonstrate any departure from normal. It may be that these differences are due to the effects of variable local or systemic factors acting upon the oral epithelium. The presence of concomitant vitamin deficiencies, smoking and hormonal changes have all been suggested as playing a role in the development of oral epithelial changes in iron deficiency (Jacobs, 1963; Jacobs and Cavill, 1968). We have used the hamster as an animal model of iron deficiency.

MATERIALS AND METHODS

Young adult male Syrian hamsters were used. Ten control animals were fed a standard normal diet and a group of 22 experimental animals were maintained on a diet containing 20 mg/kg of iron (this being approximately half the normal level). They were bled 3 times every 2 weeks from the retro-orbital sinus (Pansky *et al.*, 1961) over a period of 27 weeks. They were killed by an overdose of intraperitoneal barbiturate and the severity of iron deficiency assessed. Those with haemoglobin values of less than 12 g/dl were categorized as showing iron deficiency anaemia; animals with a percentage saturation of transferrin of less than 20 per cent but haemoglobin greater than 12 g/dl were categorized as showing iron deficiency without anaemia. The remaining animals with a percentage saturation of greater than 20 per cent but having low serum iron and nil or trace amounts of marrow iron were categorized as iron depleted.

The ventral surface of tongue was chosen as a suitable site for study. This area is readily accessible and has anatomical features allowing consistent selection of comparable sites in different animals. After animals were killed, the tongues were carefully removed by resection at the posterior reflection of the floor of the mouth. Each tongue was placed on a fresh piece of thin dental wax and, by use of a razor blade, a strip of ventral mucosa approximately 3 mm wide to one side of the midline was delineated. Using fine forceps and scissors, this strip of mucosa was removed, the portion of tissue held in the forceps discarded and the remaining tissue trimmed into 1 mm thick slices. The 1 mm slices were pulse-labelled *in vitro* with [^{3}H]-thymidine and the results of the cell kinetic investigations will be reported elsewhere.

After fixation in Bouin's solution, the material was processed in a histokine and paraffin embedded. An early section was examined to check that the epithelium was orientated at right angles to the surface. Subsequently, 3.5 μm thick sections were cut and stained by haematoxylin and eosin. Four ribbons of five sections from each block were cut and the middle section from each ribbon of five was used for the analysis.

Epithelium from the ventral surface of hamster tongue consists of three cell compartments, the progenitor compartment, the maturation compartment and the keratinized compartment (Fig. 1). Separation of progenitor and maturation compartments has to be made subjectively and the features used to differentiate between these compartments were the orientation, location and staining characteristics of the cells. Progenitor cells are smaller than mature cells, appear to have a larger nucleo–cytoplasmic ratio and are confined to the deepest cell layers. Progenitor cells are elongated at approximately right angles to the basement membrane while maturing cells appear flattened, are in the main orientated parallel to the surface and at right angles to the progenitor cells. It is somewhat simpler to distinguish the maturation compartment from the keratinized compartment. Within the keratinized compartment, there is loss of stainable nuclear chromatin, loss of cell outlines and it is not possible to distinguish keratohyaline granules.

The method used for histological quantitation was a stereological point-counting technique and is essentially that described by Eveson and MacDonald

(1978). Counting was undertaken on the screen of a teaching head of a Leitz Ortholux microscope upon which a column of known width was delineated. The section was orientated so that this column was at right angles to the epithelial surface. A transparent Perspex point-counting grid was superimposed upon the projected image and the points falling on each cell compartment were counted. The grid was rotated to 3 positions at 60° angles and repeat counts were undertaken to overcome errors due to anisotropy in the tissue and the grid. The area of epithelium in the counting column was calculated from the point counts and division of this value by the column width gave an estimate of mean epithelial thickness. Similarly, the mean thickness of individual compartments was calculated. To ensure that sufficient points were being counted for statistical purposes, the accumulative means test (Chalkley, 1943) was applied; it was found that two fields per section and four sections per animal gave an adequate number of point counts. In addition to the derivation of data which would allow the absolute values of thickness measurements of compartments to be compared, the proportions of the total epithelial thickness formed by individual compartments were calculated.

RESULTS

The results of the quantitative analyses of ventral tongue epithelium from normal and experimental animals are shown in Table 1. Comparisons between

Table 1. Mean epithelial thickness, compartment thicknesses and percentages of epithelium formed by individual compartments

Animal		Thickness in μm				Percentage of epithelium formed by each compartment		
		Progenitor	Maturation	Keratin	Total	Progenitor	Maturation	Keratin
Normal controls	1	11.0	21.0	12.3	44.3	24.8	47.4	27.8
	2	15.5	28.1	14.1	57.7	26.9	48.7	24.4
	3	11.6	21.3	14.6	47.5	24.4	44.8	30.8
	4	13.9	19.9	13.6	47.4	29.3	42.0	28.7
	5	13.6	20.4	11.0	45.0	30.2	45.3	24.5
	6	18.5	26.7	20.6	65.8	28.1	40.6	31.3
	7	13.1	20.6	13.4	47.1	27.8	43.7	28.5
	8	13.8	20.5	13.4	47.7	28.9	43.0	28.1
	9	21.1	29.4	19.6	70.1	30.1	41.9	28.0
	10	18.7	28.4	16.8	63.9	29.3	44.4	26.3
Mean		15.1	23.6	14.9	53.7	28.0	44.2	27.8
Iron depletion	1	19.5	22.3	18.0	59.8	32.6	37.3	30.1
	2	14.2	15.3	11.9	41.4	34.3	37.0	28.7
	3	20.5	31.8	18.9	71.2	28.8	44.7	26.5
	4	18.8	17.1	15.5	51.4	36.5	33.3	30.2
	5	20.5	20.9	20.5	61.9	33.1	33.8	33.1
	6	17.5	22.9	17.4	57.8	30.3	39.6	30.1
	7	15.4	18.9	16.1	50.4	30.6	37.5	31.9
	8	16.3	22.3	14.2	52.8	30.9	42.2	26.9
	9	15.5	17.4	13.3	46.2	33.5	37.7	28.8
	10	16.3	24.5	12.4	53.2	30.6	46.1	23.3
Mean		17.5	21.3	15.8	54.6	32.1	38.9	29.0
Iron deficiency without anaemia	1	18.1	27.6	20.4	66.1	27.4	41.7	30.9
	2	15.1	19.9	18.5	53.5	28.2	37.2	34.6
	3	15.1	21.8	16.0	52.9	28.5	41.4	30.3
	4	13.2	16.9	15.1	45.2	29.2	37.4	33.4
	5	19.1	25.4	19.2	63.7	30.0	39.9	30.1
	6	14.1	21.0	16.3	51.4	27.4	40.9	31.7
	7	15.4	17.8	15.5	48.7	31.6	36.6	31.8
Mean		15.7	21.5	17.3	54.5	28.9	39.3	31.8
Iron-deficiency anaemia	1	15.1	17.5	19.7	52.3	28.9	33.5	37.6
	2	13.0	17.5	17.5	48.0	27.0	36.5	36.5
	3	15.5	18.9	18.6	53.0	29.2	35.7	35.1
	4	16.1	19.8	20.9	56.8	28.3	34.9	36.8
	5	13.3	16.5	14.3	44.1	30.2	37.4	32.4
Mean		14.6	18.0	18.2	50.8	28.7	35.6	35.7

groups were made using the Mann–Whitney U test (Siegel, 1956).

In normal animals the mean epithelial thickness was 53.7 μm. The total epithelial thickness values in the groups of iron-deficient animals did not differ significantly from controls. In iron-depleted animals, the absolute values for the thickness measurements of individual compartments did not differ from control animals. However, the progenitor compartment formed a significantly larger proportion of the epithelium ($p < 0.002$) and the maturation compartment formed a significantly smaller proportion ($p < 0.02$). When the control data were compared with data from the animals showing iron deficiency without anaemia, no significant difference in the absolute values of compartments was demonstrable. The progenitor compartment formed the same proportion of the total as in controls but the maturation compartment comprised 5 per cent less of the total and this was statistically significant ($p < 0.002$). The keratinized compartment formed 4 per cent more of the total thickness ($p < 0.02$).

Comparison of the animals with iron-deficiency anaemia with controls showed no difference in the size of the progenitor compartment but the maturation compartment was significantly thinner (mean 18.0 μm as opposed to 23.6 μm, $p < 0.002$) and the keratinized compartment was significantly thicker (mean 18.2 μm as opposed to 14.9 μm, $p < 0.05$).

DISCUSSION

The results showed no change in the total thickness of the epithelium of the ventral surface of the tongue from animals with varying degrees of iron deficiency. There were, however, significant differences in the composition of the epithelium and these differences became more pronounced as the severity of the iron deficiency increased.

Anaemic animals had a significantly smaller maturation compartment than normal animals, but the reduction in this compartment did not lead to a reduced total epithelial thickness as the keratinized compartment was significantly increased in thickness. In human iron-deficient buccal epithelium, increased cornification has been shown in cytological studies (Jacobs, 1959).

Animals with iron deficiency without anaemia had no absolute change in compartment sizes but the maturation compartment formed a significantly smaller proportion of the epithelium and the keratinized compartment a significantly larger proportion of the epithelium than in normal animals. Iron-depleted animals also showed a significant reduction in the proportion of the epithelium made up by the maturation compartment, but there was a significant increase in the proportion made up by the progenitor cell compartment.

Thus, as iron deficiency develops, there is an initial increase in the proportion of the epithelium made up by progenitor cells and a decrease in the proportion formed by maturing cells. With increasing degrees of deficiency, the maturation compartment forms less, and the keratinized compartment more of the epithelium until at the stage of anaemia, there is a significant reduction in the maturation compartment and a significant increase in the keratin thickness.

There was no alteration in total epithelial thickness in anaemic animals. However, the animals were killed shortly after anaemia developed and a longer period of iron-deficiency anaemia might have produced an overall decrease in epithelial thickness. Valberg *et al.* (1961), after failing to produce any alteration in the oral mucosa of iron-deficient rats, also suggested that the duration of iron deficiency might be important. The increase in thickness of the keratin layer in anaemic animals may have disguised atrophic changes in the cellular layers. When the values for the progenitor cell and maturation compartment thickness were combined and comparisons of anaemic and control animals made, anaemic animals were found to have a thinner cellular compartment than control animals ($p = 0.07$). Although this value is not statistically significant, it lends support to the idea that atrophic changes were developing and that a longer period of iron-deficiency anaemia would result in significant epithelial atrophy.

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REFERENCES

Chalkley H. W. 1943. Method for the quantitative morphological analysis of tissues. *J. natn. Cancer Inst.* **4**, 47–53.

Drinnan A. J. 1969. Oral tissues in anaemia. *IVth Proc. of the Int. Acad. Oral Pathol.* pp. 64–82.

Eveson J. W. and MacDonald D. G. 1978. Quantitative histological changes during early experimental carcinogenesis in hamster cheek pouch. *Br. J. Derm.* **98**, 639–644.

Jacobs A. 1959. Oral cornification in anaemic patients. *J. clin. Path.* **12**, 235–237.

Jacobs A. 1960. The buccal mucosa in anaemia. *J. clin. Path.* **13**, 463–468.

Jacobs A. 1963. Epithelial changes in anaemic East Africans. *Br. med. J.* **1**, 1711–1712.

Jacobs A. and Cavill I. 1968. The oral lesions of iron deficiency anaemia: Pyridoxine and riboflavin status. *Br. J. Haemat.* **14**, 291–295.

Pansky B., Jacobs M., House E. L. and Tassoni J. P. 1961. The orbital region as a source of blood samples in the golden hamster. *Anat. Rec.* **139**, 409–412.

Siegel S. 1956. *Non-parametric Statistics for the Behavioral Sciences*, 1st edn. McGraw-Hill, London.

Valberg L. S., Taylor K. B., Witts L. J. and Richard W. C. D. 1961. The effect of iron deficiency on the stomach of the rat. *Br. J. Nutr.* **15**, 473–480.

Plate 1 overleaf.

Plate 1.

Fig. 1. Compartments of hamster ventral tongue epithelium. Haematoxylin and eosin. × 500

Plate 1.